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OENOTHERA MUTANTS WITH DIMINUTIVE CHROMOSOMES[1]

ANNE M. LUTZ

A. INTRODUCTION

For a number of years the writer has devoted considerable attention to the study of the somatic chromosomes of *Oenothera Lamarckiana* and its derivatives in an effort to ascertain whether or not each species or type is represented by a definite, fixed number of chromosomes. Having found and announced (Lutz, '12) that all forms having identical somatic characters throughout all stages of their development invariably have the same number of chromosomes, we may now consider some of the results of these studies in detail. This report may be considered the first of a series of three dealing with somatic chromosome number in mutants of the Lamarckiana group of Oenothera.[2]

During the first four years investigations were conducted at the Station for Experimental Evolution,[3] Cold Spring Harbor, where careful work on an extensive scale was made possible by the kindness and generosity of the director, Professor Charles B. Davenport.

Owing to the nature of the study, a thorough knowledge of the somatic characters of each individual became, clearly, a matter of first importance; therefore, from the time of the appearance of the seedling leaves until the end of the fruiting period late in October, attention was directed almost exclusively to the careful study of the somatic characters of the growing plants.

Tips of rapidly growing roots from rosettes in 3-inch pots, supplemented by various somatic tissues of young buds, were employed for the determination of somatic chromosome number.[4] All, with the exception of a few of the first, were fixed with chrom-acetic. One fixing fluid was employed throughout in order that reliable conclusions might be reached concerning the comparative sizes and shapes of chromosomes of various types. Fixations were made at all hours, but only those prepared between 10 A.M. and 1 P.M. offered satisfactory material for study. Tips were embedded after 5 minutes in melted paraffin and cut transversely for chromosome counts, usually about 7 μ thick. Great care was taken to preserve an unbroken series. When this was impossible, the point of interruption was carefully indicated by a diamond mark on the slide. This precaution was strictly observed, even when a single section was mounted upside down. All material was stained with Heidenhain's iron-haematoxylin.

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[1] Reported in a paper read before the Botanical Society of America, December 29, 1915.

[2] To be published in this Journal.

[3] Maintained by the Carnegie Institution of Washington until March, 1911.

[4] For the sake of brevity, the rather awkward term of "chromosome number" will be substituted frequently for "number of chromosomes" throughout this report and others to follow.

The chromosome numbers of mutant types to be announced by the writer in the papers of this series, as well as those of forms mentioned in previous reports (with very few exceptions, notable among which is *O. gigas*), were determined from fixations prepared from the mutant, and not from offspring of the mutant duplicating the characters of the parent, although counts were also made from these when the opportunity was presented.

In order to be assured of the correct identification of the Cold Spring Harbor mutants supposed to duplicate the characters of forms which de Vries had described and named, I visited Professor de Vries's gardens at Amsterdam in July and September, 1911, and in June, 1912, where, through the courtesy of Professor de Vries, I had the privilege of studying forms of particular interest as greenhouse rosettes, and, later, as flowering plants. The distinguishing characteristics of important forms were carefully noted and later compared with descriptions and photographs of Cold Spring Harbor types supposed to duplicate them.

From time to time, Professor de Vries has greatly assisted in the elucidation of obscure points by furnishing the writer with careful and detailed descriptions of certain forms whose identity, on American soil, had not been clearly established; by giving additional information, when perplexing questions arose, regarding certain forms and cultures mentioned in his publications; and by providing the writer, on many occasions, with generous supplies of seeds of important and significant forms. It is with the keenest sense of my obligations to Professor de Vries that I express my gratitude for his never-failing kindness.

It was my highly esteemed privilege to compile the results of the

Cold Spring Harbor studies and to work out the majority of the details of special cell-study connected with these investigations (exclusive of chromosome counts) in consultation with Professor V. Grégoire at the University of Louvain during the year 1911–12. It is with the most profound gratitude that I express my appreciation of the help received from Professor Grégoire in this work—a portion of which is represented by these three communications; of his untiring devotion of time and thought from October, 1911, until August, 1914, to the correct interpretation of recorded observations; to the verification of numerous microscopical details of the work; and to the solution of many problematical questions which have arisen in connection with these studies. The chromosome count of every type mentioned by the writer in this and the two reports to follow, was carefully verified by Professor Grégoire, especial attention having been given to those which for any reason might be questioned. It is reasonably certain, therefore, that the identification of the Cold Spring Harbor forms and the chromosome numbers to be announced in the three papers of the series, are correct.

To Professor J. C. Arthur, Professor Stanley M. Coulter and Mr. George N. Hoffer, I am most deeply indebted for innumerable courtesies and many privileges enjoyed in the botanical laboratories of Purdue University during the past three years.

I wish also to express my gratitude to Professor H. E. Crampton for privileges accorded to me, through his courtesy, at the American Museum of Natural History; to Dr. F. E. Lutz and to Professor B. M. Davis for numerous favors and accommodations; to Professor H. H. Bartlett for generously providing me with unpublished data relating to certain new and interesting forms which he has recently discovered, and for his kindness in giving me permission to quote his statements concerning them in these communications.

The primary object of these three papers is to discuss, in the light of the Cold Spring Harbor and Louvain studies of somatic chromosome number in *Oenothera Lamarckiana* and its derivatives, certain theories and conclusions which Gates has announced from time to time, and which Gates and Miss Thomas have based upon the results of their investigations. Partly for this reason, but chiefly because of limitations of space, all but a few of the more important mutants reported will be described very briefly, or not at all, these descriptions being reserved for a later publication. With the exception of a few forms which are of particular interest, those which have appeared but once or twice or which have appeared more frequently, but in every case have failed to come to flower, will be designated temporarily by type numbers only.

It may be understood that all facts and theories embodied in the reports of this series pertain only to the plants of the *Lamarckiana* group, unless otherwise stated.

As a preliminary to the study of 14+-chromosome forms, we may briefly consider the 14-chromosome mutants which have appeared in the Cold Spring Harbor cultures.

B. 14-CHROMOSOME MUTANTS

Five hitherto undescribed types, whose chromosome numbers have not been previously announced, appeared in cultures of *O. Lamarckiana*, *O. lata*, *O. nanella*, etc. All were found to have 14 chromosomes.

(1) Type 2787 and (2) type 2803 are unimportant forms and may be passed by for the present.

(3) Type 3539, found in a 1908 culture of *O. Lamarckiana*, had narrow, asymmetrical leaves, a character which persisted throughout life. The majority of the flowers had 4+-rayed stigmas. The somatic chromosome number of this plant was ascertained in 1909.

(4) *O. plicatula* appeared among the offspring of a selfed *lata* mutant in 1909. It was very beautiful and thrifty in appearance. Coming to flower as an annual, it attained a height of about 9 dm. when full grown. As in the case of *O. Lamarckiana* and other forms, a circlet of rosette branches was given off (text fig. 1), those of the mutant ascending more rapidly than the rosette branches of *O. Lamarckiana*. The buds were quadrangular, the sepals attaining the deep red of *rubrinervis*. The open flowers measured about 7–8 cm. in diameter, as a rule, and readily attracted the eye by the peculiar markings shown in the accompanying photographs (text figs. 1 and 2; compare the latter with a flower from *O. Lamarckiana*, text fig. 3). These troughs or ridges, as they may be called, according to the surface of the petal from which they are viewed, result from a somewhat too snug fit of the quadrangular cone of petals within the quadrangular cone of sepals. Since the distance between the angles of neighboring sepals appears to be less, in such instances, than the distance between the angles of the petal

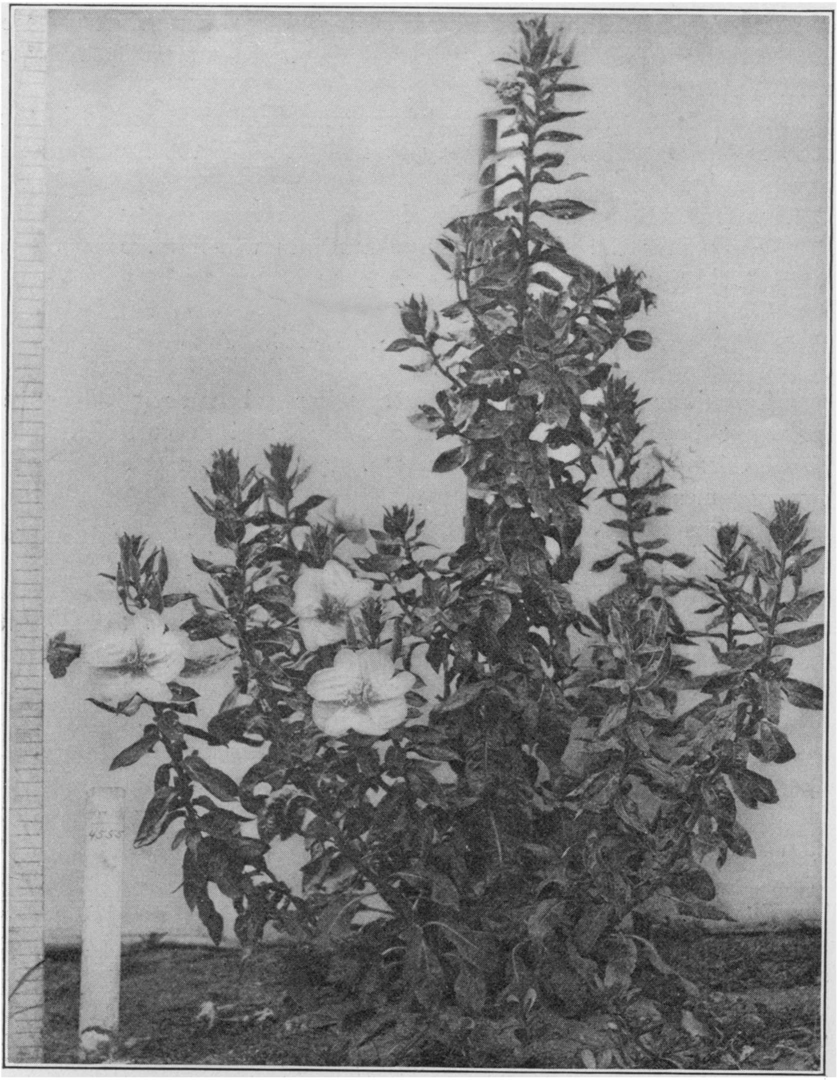

FIG. 1. *O. plicatula*, plant No. 4555, C. S. H., 1909. Mutant offspring of *O. lata*, selfed. Photographed August 25, 6:40 A. M. Flowers with "crimped" petals.

cone directly beneath, the petals are pushed into a sort of trough (whose convexity must appear upon the stigma surface of the petals) extending in the same direction as the line marking the union of two neighboring sepals.

Markings of this sort, though less pronounced and often more irregular in appearance, are found in the newly-opened flowers of many forms, but they usually disappear shortly after the petals are released. The crimps or ridges in the petals of *O. plicatula* persisted as shown in text figure 2 until the flowers faded. The flowers shown in text figures 2 and 3 were photographed in the early morning of the same day.

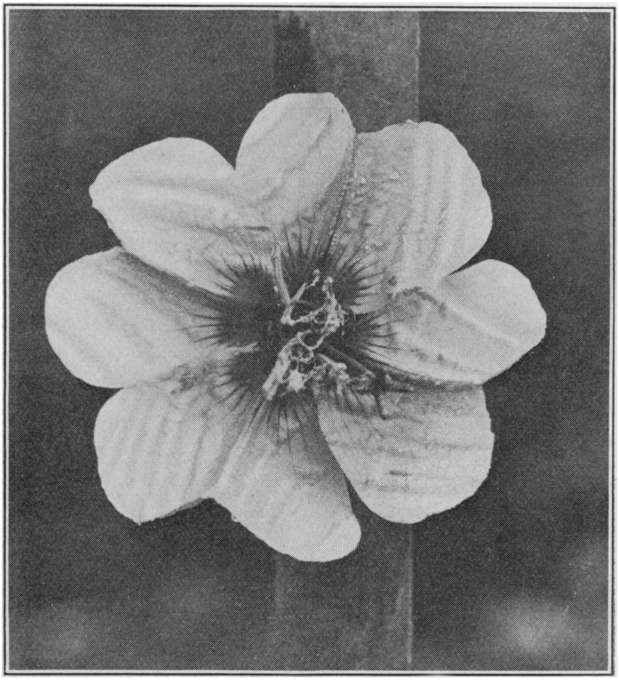

FIG. 2. *O. plicatula*; flower taken from plant shown in Text-fig. 1. Photographed September 19, 6:40 A. M., 1909. Showing "crimped" petals.

While *O. plicatula* could not be mistaken for *O. rubrinervis* under any circumstances, a type grown at Cold Spring Harbor which may have been identical with de Vries's *rubrinervis*, had crimped petals and red sepals (text fig. 4).[5] In 1913 a few plants were grown from seeds of the Amsterdam *rubrinervis*, kindly sent to me by Professor de Vries, and the single individual which came to flower had crimped petals.

O. plicatula produced an abundance of seemingly good pollen consisting of 3-lobed grains. Unfortunately, no effort was made to self this mutant.

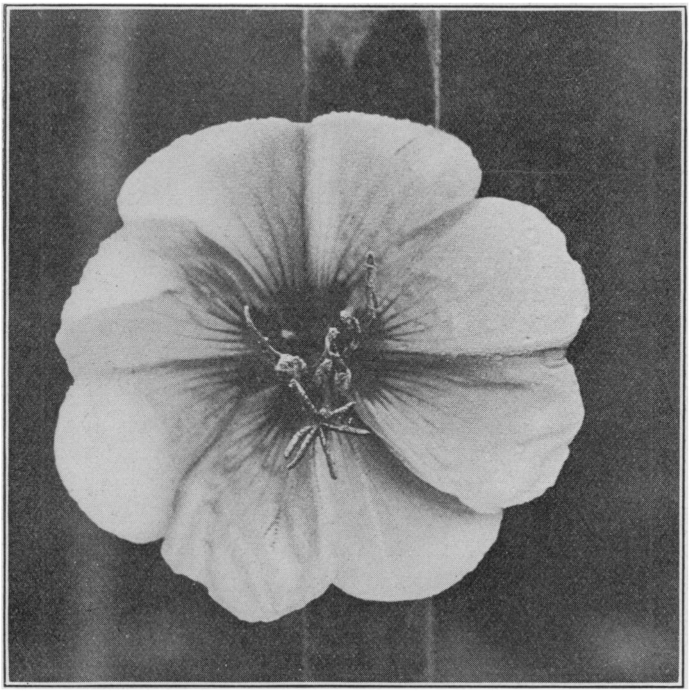

FIG. 3. *O. Lamarckiana*, plant No. 4650, C. S. H., 1909. Offspring of *O. lata*, selfed. Photographed September 19, 6:50 A. M., same day and hour as flower shown in Text-fig. 2. Showing absence of "crimps" in petals.

(5) *O. delicatula*, a form of especial interest, will be fully described in a later communication.

(6) Type 3514 (described on page 520 of this report) may be a modified form of de Vries's *rubrinervis*. While the chromosome number of this form has not been announced previous to this communication (un-

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[5] This character is much more pronounced in some individuals than in others.

less the *rubrinervis* in which Gates found 14 chromosomes duplicated this type instead of de Vries's mutant), the type is not a new one, having been figured by MacDougal as early as 1905.

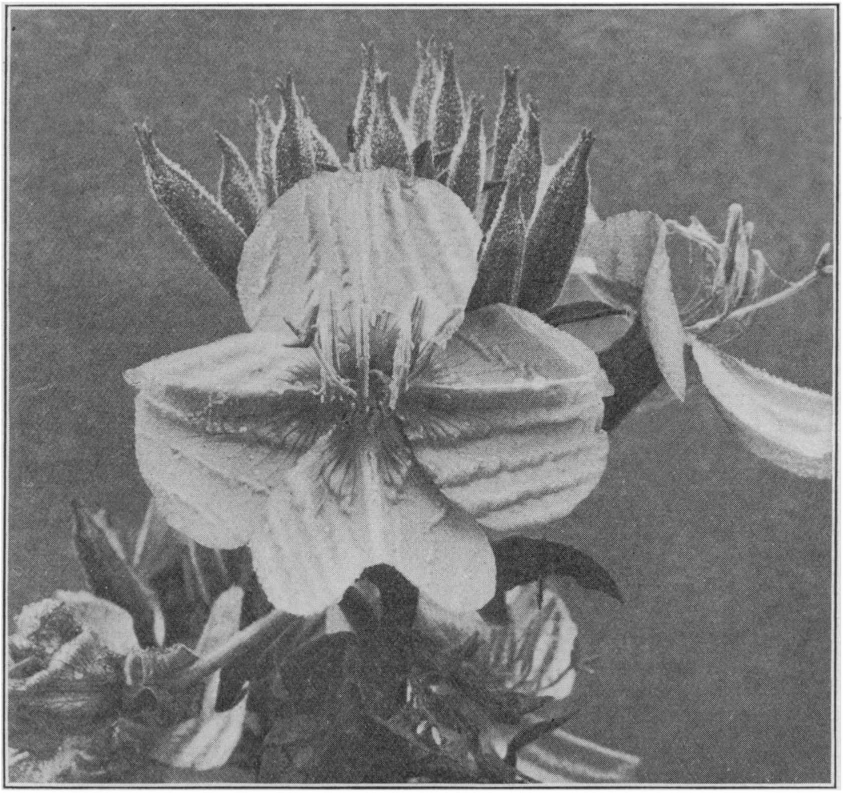

FIG. 4. *O. rubrinervis* (?), plant No. 3903, C. S. H., 1908. Flower photographed in early morning, showing "crimped" petals.

C. 14^{+1}-CHROMOSOME MUTANTS

1. *Recent discoveries indicative of the production of 7^{+1}-chromosome gametes by 14- and 15-chromosome forms*

The discovery of chromosome degeneration in 14+-chromosome forms by Geerts ('11), together with later studies of this peculiar irregularity in 15-chromosome forms by Gates and Miss Thomas ('14), has thrown a flood of light upon the origin of the small chromosome in 14^{+1}-chromosome offspring of 15-chromosome *O. lata* × 14-chromosome *O. Lamarckiana.*

Chromosome degeneration was observed by Geerts in 21-chromosome offspring of *O. lata* × *O. gigas*, *O. Lamarckiana* × *O. gigas* and *O. gigas* × *O. Lamarckiana,* all from de Vries's gardens. On the heterotypic spindles of the individuals which he studied he found 7 pairs of whole chromosomes and 7 which were unpaired. With the first division the two members of each of the 7 pairs separated and passed to opposite poles, while 3 of the 7 unpaired members of the group usually moved towards one pole and 4 towards the other. As the 7 chromosomes approached the pole the longitudinal split, preparatory for the second division, became quite distinct, but was less clear and more irregular in the 3 or 4 remaining chromosomes. Sometimes, also, the latter failed to reach the pole and were not included within the daughter nucleus. Later, 7 clearly split chromosomes and 3 or 4 small ones showing the longitudinal split less clearly, were found at the equator of each of the two homotypic spindles. Thus, to each of the four poles were distributed 7 distinct chromosomes and often a number of irrgeular chromosomes or pieces of chromosomes. In regard to the fate of these fragments or irregular bodies, he says (italics not employed in the original): "*Wenn die Tetradenkerne entstehen, wird bisweilen dieses Chromatin in den Kern aufgenommen,* aber meistens liegen ausserhalb der Kernwand auch Chromatinteilchen. Diese Chromatinstücke, welche ausserhalb der Kerne zurückgelassen werden, entwickeln sich oft zu Zwergkernen, in den jungen Pollenkörnern sowie in dem jungen Embryosacke. Beim Auswachsen des Embryosackes verbleichen diese Zwergkerne und verschwinden allmählich."

From Gates and Miss Thomas (pp. 538–539) we learn that ". . . in the heterotypic mitosis in the *lata* and *semilata* forms, the 15 chromosomes are usually distributed so that 7 go to one daughter-nucleus and 8 to the other. In rare cases the distribution is 9 and 6. . . . More frequently the extra chromosome is left behind, where it may be seen fragmenting and degenerating in the cytoplasm."

A remarkable irregularity in 15-chromosome *O. lata rubricalyx* is described by Gates and Miss Thomas (pp. 541–542) and the statements which follow are of particular interest in view of the fact that the Cold Spring Harbor and Louvain studies have demonstrated the actual existence of 14^{+1}-chromosome mutants:

"The left-hand group contains 7½ chromosomes, while the right-hand group consists of 6 whole chromosomes, a half-chromosome and a small fragment. There is in addition a chromosome in the cytoplasm near this group, which has been left out of the nucleus in interkinesis. This makes the full quota of 15 chromosomes. The origin of the fragment is obscure, but the figure shows that even fragments of chromosomes may be distributed occasionally to the daughter-nuclei. Unless afterwards extruded from the pollen nuclei, such fragments will no doubt affect the later development of the individual; they might remain independent or become attached to or fused with one of the other chromosomes."

Thus the researches of Geerts have shown that during the female, as well as male, reduction in certain 21-chromosome forms, chromosomes in excess of 14 may fragment and degenerate, while the researches of Gates and Miss Thomas have demonstrated that one of the chromosomes of certain 15-chromosome forms may fragment and degenerate during the reduction divisions of the pollen mother cell. These facts, together with other evidence which will be offered by the writer later, suggest the conclusion that *chromosome degeneration occurs in all forms having 14+-chromosomes, though probably less frequently in plants having 28, than in other 14+-chromosome forms particularly if the 28 represent a double set of the original 14. In other words, there appears to be a tendency in 14+-chromosome forms, to return to the original 14-chromosome condition; furthermore, this tendency appears to be more pronounced in forms having 21 chromosomes, than in those having a double set of the original 14.* It may be that sometimes a portion, sometimes all, of the chromosomes in excess of 14 degenerate, and that gametes of 14+-chromosome plants are equipped with various irregular numbers of chromosomes.[6] In addition to the whole 7 or 7+-chromosomes present in the germ-cells of such forms, we may expect to find, occasionally, one or more fragments of chromosomes.

2. *14^{+1}-chromosome offspring of O. lata × O. Lamarckiana*

A 14^{+1}-chromosome condition has been observed by the writer in 3 plants. The first, No. 3878, was found among the F_1 offspring of *O. lata* × *O. Lamarckiana* grown at Cold Spring Harbor in 1908 and the second, No. 4605, in another culture of the same cross in 1909.

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[6] Doubtless more frequently true of 21-, than of 15- and 28-chromosome forms.

The second mutant duplicated the vegetative characters as well as the somatic chromosome number of the first; therefore the type represented by these two individuals will be known as *O. aberrans*.

O. aberrans (text fig. 5) attained the height of *O. Lamarckiana*, thereby exceeding the height of the female parent. It produced a number of long rosette branches which were somewhat more decumbent than those of *Lamarckiana*. The buds, which remained yellow throughout the entire season, were tapering and slender. A large number of flowers were produced daily, 86 having been counted on No. 4605 one

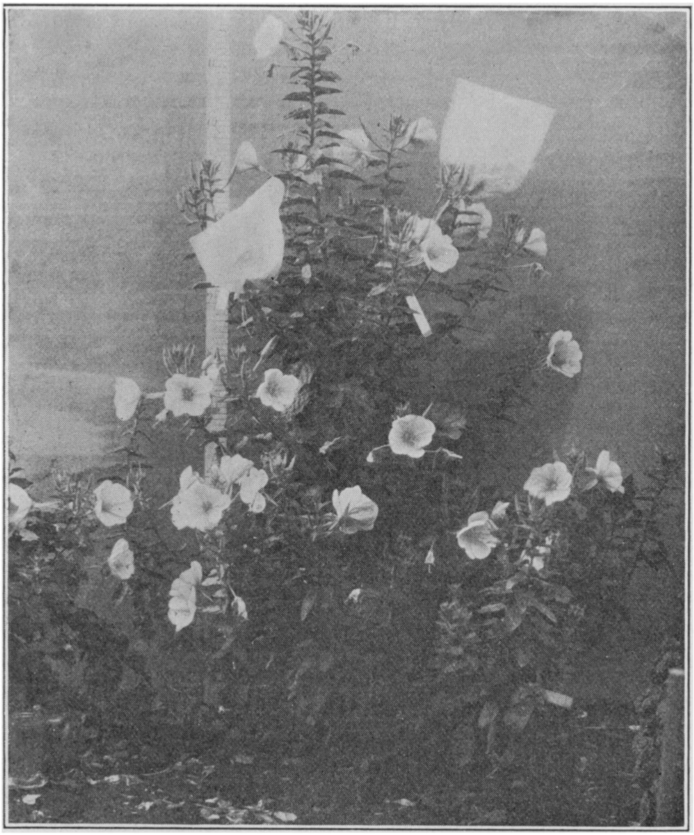

FIG. 5. *O. aberrans*, plant No. 3878, C. S. H., 1908. 14^{+1}-chromosome mutant offspring of *O. lata* × *O. Lamarckiana*.

day near the height of the flowering season. The petals were smooth and a slightly lighter yellow than is characteristic of *O. Lamarckiana.* A portion of the buds produced a moderate abundance of pollen containing relatively few seemingly good grains and still others none at all. It was almost impossible to obtain seed from the self-pollination of this form.

The somatic chromosomes of the 1908 mutant (No. 3878) were studied and the number determined in the autumn of 1909. Finding that this plant had 14 chromosomes of the usual size and 1 small one, it was recalled that a second mutant (No. 4605) of the same type had been produced by *O. lata* × *O. Lamarckiana* in the season which had just passed; therefore root-tips from this plant were sectioned and studied with much interest, and here also 14 chromosomes of the usual size and 1 small one were found (late autumn of 1909).

Unfortunately, but one fixation of 16 tips had been prepared from No. 3878 and one of 20 tips from No. 4605. These were not wholly satisfactory for study, as the fixations were poor and only comparatively few figures were found in perfect metaphase (the only satisfactory stage for the determination of chromosome number in root tips of *Oenothera*). However, the tendency of the small chromosome to occupy a peripheral position on the equatorial plate and to be alloted the full space of a large chromosome, often made it possible to determine the presence of the small member when it was impossible to count the long ones.

In transverse sections of root-tips from No. 4605, 25 metaphase figures were observed, showing the small chromosome with unmistakable clearness. In but 8 of this number were the large chromosomes sufficiently well separated to make it possible to count them accurately, but in each of the 8, 14^{+1} were clearly demonstrated (figs. 2 and 3).

In No. 3878, 59 figures were observed in which the small chromosome was clearly identified. In 30 of the 59 the chromosomes were sufficiently well separated to enable one to determine the number precisely, and in each of the 30, 14^{+1} were counted (fig. 1).

Although in both plants the small chromosome occupied a peripheral position on the equatorial plate in the majority of cases (figs. 1 and 3), its position was by no means constant, as it was found farther in occasionally (fig. 2).

In 1911 and 1912 I had the privilege of preparing fixations for the determination of somatic chromosome number from many interesting forms in Professor de Vries's garden at Amsterdam. One of these, No. 6082, was a mutant in a *lata* × *Lamarckiana* culture identified by Professor de Vries as a typical *O. rubrinervis*. Lutz ('07)[7] and Gates ('08) had reported 14 chromosomes for *rubrinervis;* it was a matter of considerable surprise, therefore, to find 14 chromosomes of the ordinary size and 1 small one in the root-tips of this plant. Notwithstanding the fact that but one fixation of 13 tips had been prepared from de Vries's *rubrinervis*, these chanced to be exceedingly well preserved and to show an abundance of good figures in metaphase; therefore the somatic chromosomes of this plant were studied with the greatest care by Professor Grégoire and myself in 1912.

A total of 125 metaphase figures showed the small body distinctly, although the longer ones were too massed and tangled to count with accuracy. In 52 figures 14 chromosomes of the usual size and 1 small one were distinctly counted. As in the preceding forms, the small body usually occupied a peripheral position on the equatorial plate and was commonly alloted the full space of a large chromosome (figs. 4 and 5. See also figs. 1, 2, 3). It was sometimes found farther in, however, and its position in the group was by no means constant. Usually, the small body stood out with remarkable clearness, but not infrequently the chromosomes were too massed and tangled to determine whether or not the small body was present. Sometimes it appeared to be absent, but careful focusing revealed it lying very close beside one of the longer chromosomes or in the apex of a V, formed by contact of the ends of two chromosomes. In several cells of each of the three plants the long chromosomes appeared to be sufficiently well separated to reveal the small body, had it been present, yet it was not found. In such cases it may have been absent, hidden by one of the longer chromosomes, or torn out of position by the knife in sectioning. Such figures were comparatively rare, however, and the large number of cases in which the small body was recognized in groups of tangled chromosomes where one would expect it to be hidden, was one of the striking features of these plants.

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[7] In a paper read at the Seventh International Zoological Congress in 1907 (published in the Proceedings of the Congress in 1910) the writer announced 14 chromosomes for *O. rubrinervis* offspring of open-pollinated *O. rubrinervis*. It is now well known that, in addition to the mutants *O. lata*, *O. oblonga*, *O. rubrinervis*, etc., *O. Lamarckiana* and certain other forms produce *lata*-like, *oblonga*-like, *rubrinervis*-like, etc., mutants which may be readily mistaken for the true types by inexperienced workers. Later cultures have shown that several of my first season's identifications (1907) were erroneous ones of this sort (see second paper of this series). Since the "*rubrinervis*" offspring above referred to were grown in 1907, the possibility of error in identification is conceded. Seeds of *O. rubrinervis* de Vries were received from Professor de Vries in 1913, but having in my possession no seeds of the strain from which the 1907 parent was derived, I have been unable to compare the two forms directly. The original "*rubrinervis*" mutant was produced at the New York Botanical Garden. Re-examination of the sections of root-tips from which the original counts were made revealed many beautiful metaphase figures with 14 chromosomes, but none with 14^{+1}.

3. *Origin of the Small Chromosome*

Gates and Miss Thomas occasionally found chromosomes fragmenting and degenerating on the heterotypic and homotypic spindles of 15-chromosome forms. Sometimes, also, a chromosome of the heterotypic group in a 15-chromosome form was seen to be pulling irregularly apart in the middle, leaving strands of chromatin matter trailing between the separating portions. The same peculiarity was observed in a 14-chromosome *Lamarckiana*-like offspring of a 15-chromosome plant. The authors believe that this indicated a pathological condition of the chromosomes concerned and that probably they would have degenerated later.

The two 14^{+1}-chromosome plants having identical vegetative characters (*O. aberrans*, Nos. 3878 and 4605) had the same type of small chromosome, namely, a body which was quite short and even more slender, it sometimes appeared, than the long members of the group. On the other hand, the 14^{+1}-chromosome *O. rubrinervis* had a different type of small chromosome—a body which was no longer than the small chromosome of the two preceding plants, but which seemed to be even broader than the other members of the group, at least in some instances. These conditions are particularly interesting, in view of the fact that these two mutant types were strongly contrasted in so many of their vegetative characters and were about as unlike as it is possible for two forms to be.

The small body of these two types of 14^{+1}-chromosome mutants may have arisen in the same or in a different manner. One or both may have represented merely a fragment of a degenerating chromosome, such as Gates and Miss Thomas observed on heterotypic and homotypic spindles, or a remnant of one of the two halves of a heterotypic chromosome which had pulled apart in the middle. It is even conceivable that the slender body of *O. aberrans* may have been merely a remnant of a trail of chromatin matter or a remnant of one of the very interesting "half-chromosomes" which they describe—the half of a chromosome which has separated from its mate on the heterotypic, instead of the homotypic, spindle, and which the authors believe degenerate later, in many cases. The small body in de Vries's *rubrinervis* may have been merely a piece, which by some process had become detached from one of the long chromosomes. This raises the question, Have we any evidence to indicate that pieces sometimes become detached from chromosomes?

Gates and Miss Thomas state (p. 537) that several cases were found in one of nine 15-chromosome plants having *lata* or *semilata* characters "in which the somatic chromosomes at metaphase were more or less completely divided transversely into two." They refer to Agar's studies of this phenomenon in Lepidosiren in which it was found that the chromatin matter only, and not the linin of the chromosomes, segmented. Their assertion that certain somatic chromosomes of one of their 15-chromosome plants were "more or less completely divided transversely into two," fails to give the reader a precise idea of the conditions which they observed. By "more or less completely" do they mean that some were completely divided into separate parts and others only partially so? Their meaning is obscure.

During the year spent in Professor Grégoire's laboratory especial attention was given to the study of these chromosomes and ample evidence was found to indicate that these supposed segments, at least in the majority of cases, were merely whole chromosomes with clear spaces, or unstained regions. As Gates and Miss Thomas state, these light areas were sometimes observed near the middle, and sometimes nearer one end (figs. 7, 8, 9, 10). Often, also, two such areas were found in one chromosome, giving the body the appearance of having divided transversely into three parts (fig. 10*c*). Frequently, clear spaces were found in two or more chromosomes of a group (fig. 9), but I recall no instance in which all displayed this peculiarity. In heavily stained material it is often possible to detect the delicate side lines connecting these supposed segments of chromosomes, thus proving that in these particular cases at least, we are dealing with whole chromosomes (figs. 10*a*, 10*b*, 12, 13). In extracting the stain (iron-haematoxylin) from material designed for chromosome studies the color is usually removed entirely from the linin; as a result, it is impossible to detect the linin connections between the two heavily stained portions of the chromosome (*i. e.*, the linin surrounding the unstained or clear area) and the observer is led to conclude that actual and complete transverse segmentation has occurred. However, if one examines these segments in somatic metaphase, it will be apparent, in the majority of cases at least, that these supposed segments lie in line with each other. (See figs. 7, 8, 9.) If the observer will connect the supposed segments with delicate side-lines and will then fill in the inclosed clear areas with soft crayon, he will usually find that he has a perfectly normal-appearing whole chromosome and that this is true whether the supposed segments are curved or straight. One occasionally finds these darkly stained portions entirely disconnected and out of line with each other (fig. 11), but there is generally plenty of evidence to show that they have been roughly torn apart by the knife in sectioning. Nuclear prophase chromosomes with clear areas are very frequently encountered (fig. 6). If such bodies are indeed whole chromosomes and not segments of chromosomes, then we should find corresponding halves of such chromosomes in anaphase, and these are shown in figs. 12 and 13.

These clear areas may indicate points at which the chromatin matter has undergone some change whereby it becomes incapable of taking the stain, or of retaining it, when the section is extracted for chromosome study; it is also possible that these regions are empty spaces resulting from shrinkage of the chromatin matter following fixation. The fact that chromosomes are occasionally torn apart through this region in sectioning, is certainly indicative of weakness at this point.

The above facts are related merely to show that owing to the complete extraction of stain from the linin surrounding clear areas in chromosomes, one may be led to conclude that a chromosome has actually divided transversely into two parts when it has not. However, the writer has not attempted to prove that chromosomes never break apart through these regions, for it is possible that they do, in rare instances.

In *Oenothera*, so far as I am aware, chromosomes with clear areas have been found in somatic tissues only. Geerts and Gates and Miss Thomas do not mention having observed this peculiarity in any of the chromosomes of the generative cells of 14+-chromosome forms which they studied. Furthermore, if we regard the appearance of these unstained regions in somatic chromosomes merely as a stage preceding fragmentation, then we should find numerous instances of fragmentation in groups of somatic chromosomes, but we do not.

While the plant in which Gates and Miss Thomas found somatic chromosomes with clear areas chanced to be a 15-chromosome mutant, it should not be inferred that this peculiarity is characteristic of 15-chromosome forms only, nor even of 14+-chromosome forms only. *It is not associated with the 14+-chromosome condition in* Oenothera, *for these chromosomes are just as common in 14-chromosome* O. Lamarckiana *and other 14-chromosome forms as in 14+-chromosome individuals.* I have found plants (mutants and hybrids) in the *Lamarckiana* group with the following somatic chromosome numbers; 14, 14^{+1}, 15, 16, 18, 19, 21, 22, 23, 24, 26, 28, 29 and 30—the number being constant, so far as ascertained, for each type and for each individual of the type—and have observed these chromosomes with clear spaces in every type studied. It was found that this peculiarity was no more common in hybrids and in mutants than in *O. Lamarckiana*, nor more common in 14+- than in 14-chromosome forms.

Since each of the three 14^{+1}-chromosome plants had a 15-chromosome mother (♀ *O. lata* × ♂ *O. Lamarckiana*) it is probable that the small chromosome was derived from the mother, and that each individual was the product of ♀ 7^{+1} + ♂ 7. However, Gates and Miss Thomas found that the extra chromosome of 15-chromosome forms degenerated only occasionally; furthermore, since they found chromosomes pulling apart in the middle and sometimes "fragmenting into three parts" in 14- as well as in 15-chromosome plants, it is conceivable that *O. lata* × *O. Lamarckiana* might produce a 14^{+1}-chromosome offspring by means of a ♀ 8 + ♂6^{+1} union. If the small body of de Vries's *rubrinervis* was merely a piece which had become detached from one of the ordinary chromosomes, then it may have been derived either from the 15-chromosome mother or the 14-chromosome father (♀ 7^{+1} + ♂ 7 or ♀ 7 + ♂ 7^{+1}). The small body may have been derived from the 14-chromosome parent by still another means. Since the heterotypic chromosomes of *O. Lamarckiana* are occasionally distributed in groups of 6 and 8, it is quite possible that one of the members of the 8-chromosome group sometimes fragments and degenerates just as it does at times in the 8-chromosome groups of 15-chromosome forms.

Should a fragment of the 8th chromosome chance to persist, a 7^{+1}-pollen grain might be formed.[8]

Thus far, no 14^{+1}-chromosome offspring of *O. Lamarckiana* has been reported; hence it is probable that the small chromosome of the three 14^{+1}-chromosome mutants was derived from the *lata* mother in each case. Whatever its origin, as a somatic body, it is capable of dividing longitudinally and of performing other normal activities of ordinary chromosomes during cell division.

4. *Fate of the Small Chromosome in Succeeding Generations*

During the years in which this work was conducted at the Station for Experimental Evolution, Dr. G. H. Shull kindly gave me the privilege of studying all forms of interest which appeared in his cultures of *Oenothera*, and it was among his *lata* × *Lamarckiana* hybrids that No. 3878 appeared in 1908. With perseverance I succeeded in obtaining a few seeds from this plant, selfed, and the offspring were grown by Dr. Shull in 1909. However, since the somatic chromosome number of the parent was not determined until late autumn of that year, the importance of determining the chromosome numbers of the offspring was not appreciated until the season had passed, consequently no fixations were prepared and no records of the vegetative characters of these plants are in my possession. The fate of the small chromosome is, therefore, unknown.

Did a portion or all of the offspring of No. 3878, selfed, have 14^{+2}-chromosomes? Since Geerts found that 7 whole (male and female) chromosomes of 21-chromosome forms may be eliminated and Gates and Miss Thomas have shown that 1 or more of the ordinary (male) chromosomes of 15-chromosome forms may fragment and degenerate, it is probable that the small chromosome of 14^{+1}-chromosome forms is eliminated in a similar manner, in many cases, at least. If a portion of the male and female gametes had 7^{+1}-chromosomes and the remainder 7, then it is possible that 14-, 14^{+1}- and 14^{+2}-chromosome forms were produced.[9] We know that 14-, 15- and 16-chromosome forms never have duplicate vegetative characters; would the same be true of 14-, 14^{+1}- and 14^{+2}-chromosome plants?

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[8] Likewise, it is possible that 7^{+1}-chromosome female gametes are occasionally produced in this manner by 14-chromosome forms. All facts considered, it is more probable that 7^{+1}-chromosome female gametes, capable of functioning, than that 7^{+1}-chromosome male gametes, capable of functioning, are produced by *O. Lamarckiana* and other 14- or 15-chromosome forms.

[9] If 7^{+1}-chromosome gametes are produced by 14^{+1}-chromosome forms, it is probable that they are unisexual.

5. *Chromosomal Individuality*

Gates found that the chromosomes of *Oenothera* present no constant differences in size and shape, and, with the exception of the small chromosome in the 14^{+1}-chromosome mutants, the observations of the writer are in full accord with those of Gates. The small member of the 14^{+1}-chromosome group, however, presents a very conspicuous exception, for we have seen that *it may be recognized constantly by its shape and by its diminutive size.* It furnishes very strong evidence in support of the theory of chromosomal individuality.

6. *O. rubrinervis*

While de Vries has shown that *rubrinervis* is one of the common mutant types produced by *O. Lamarckiana,* I have found no mutant in the Long Island or Indiana cultures of *O. Lamarckiana* × *O. Lamarckiana* which could be said to duplicate the type which de Vries has figured and described ('09, Vol. I, figs. 49 and 67). However, a 14-chromosome mutant (type 3514, see page 508) which seems to be a modified form of *O. rubrinervis* de Vries, is very common. It differs from the Amsterdam plant chiefly in height and branching habits, attaining the full height of *O. Lamarckiana,* and, like the latter form, giving off a number of long rosette branches. It is shown as an early flowering plant in text figure 6 and as a late flowering plant in text figure 7. MacDougal's excellent photograph ('05, Pl. XXI) of "*O. rubrinervis*" at the New York Botanical Garden portrays the branching habits of the Cold Spring Harbor mutant very clearly. Since it appears to duplicate the vegetative characters of the C.S.H. plant, it is probable that it also had 14 chromosomes. In response to an inquiry concerning the history of this individual, Dr. MacDougal stated that the plant photographed had been grown directly from seeds received from Professor de Vries.

Type 3514 produced quadrangular buds with deep red sepals; the flowers also were deeper yellow than *Lamarckiana* flowers. Large quantities of seemingly good pollen were exposed, consisting of 3-lobed grains, and seeds from these plants, selfed, were obtained without difficulty.

In the spring of 1913 Professor de Vries very kindly provided me with seeds of the Amsterdam mutant. These were sown in March of that year, but owing to drought, late transplantation and a destructive attack of aphids, which showed a curious preference for

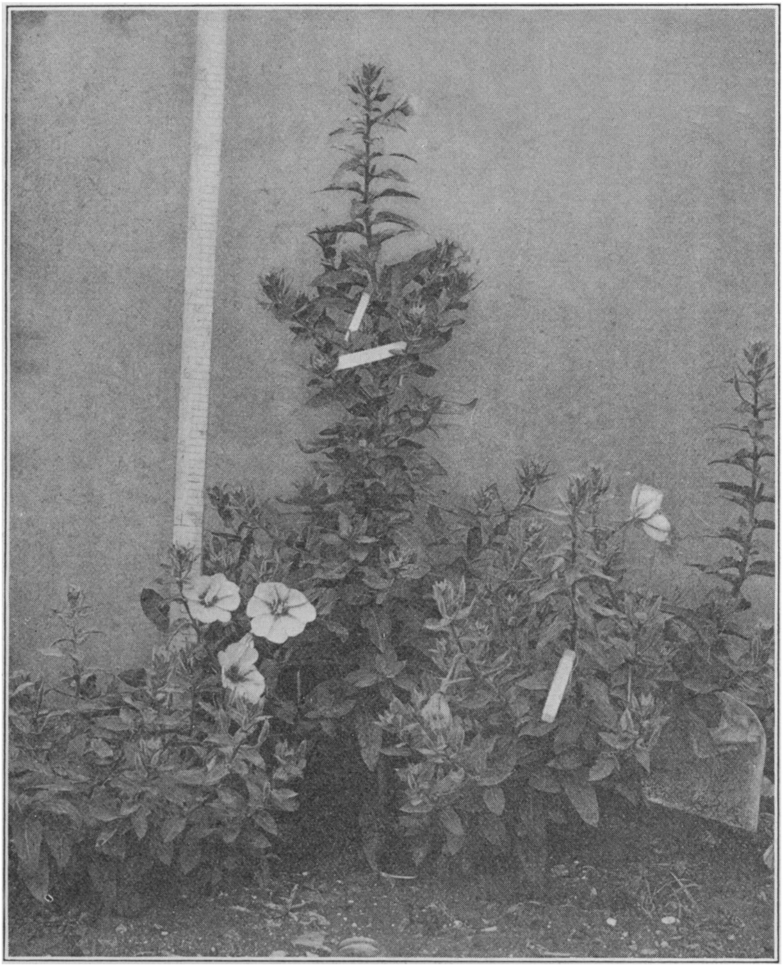

FIG. 6. Type 3514, plant No. 3514, C. S. H., 1908. Mutant offspring of *O. Lamarckiana*. Supposed to be a modified form of de Vries's *rubrinervis*. Photographed during early flowering period.

rubrinervis, only one plant came to flower. This individual corresponded perfectly with de Vries's descriptions and photographs of *rubrinervis*, but did not duplicate the C.S.H. type, though it had many characters in common with it. Their resemblance was noted in the

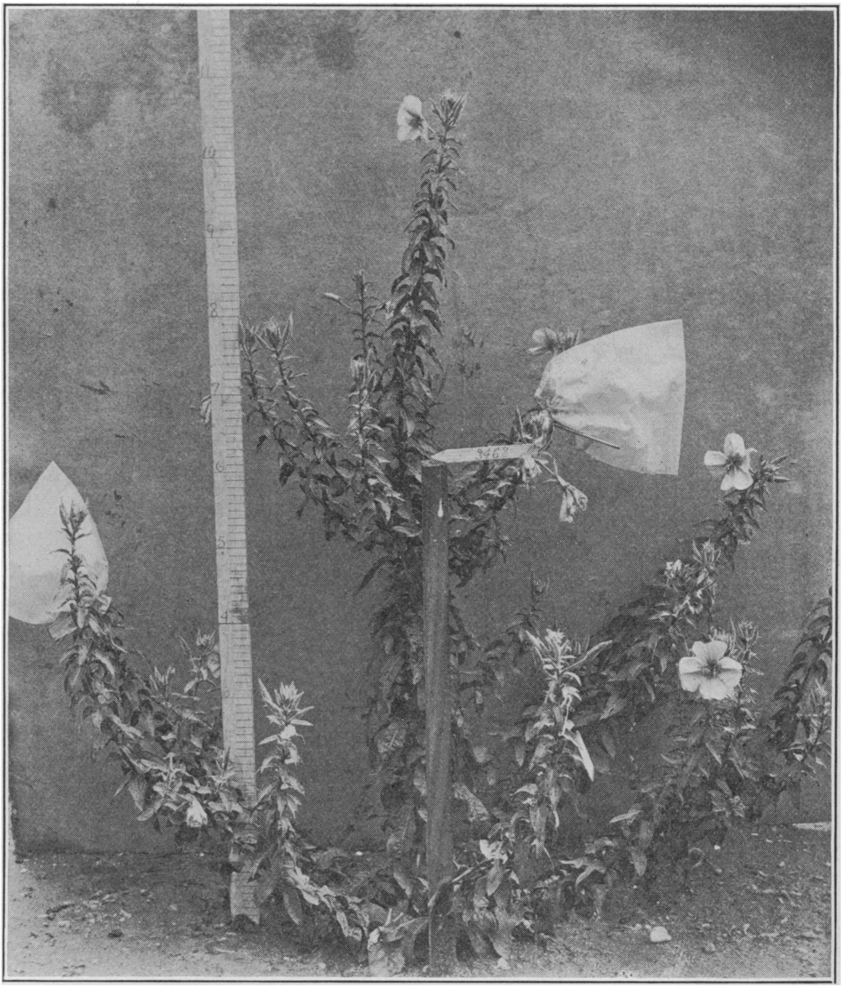

FIG. 7. Type 3514, plant No. 3464, 1908. Mutant offspring of *O. Lamarckiana*. Late flowering period.

striking similarity of the young rosettes, in the red sepals, deep yellow petals, quantities of seemingly good pollen produced, long seed-capsules, color and shape of the leaves. They differed chiefly in height and branching habits. If the reader will compare the C.S.H. and New York Botanical garden type with de Vries's photographs, their dissimilarity with respect to the latter character will be apparent at once. The C.S.H. mutant is found in cultures of *O. Lamarckiana* × *O. Lamarckiana* (also among offspring of *O. lata* × *O. Lamarckiana* and *O. lata*, selfed), while it is quite possible that de Vries's *rubrinervis* is produced by selfed *Lamarckiana*. If one is inclined to believe that de Vries's *rubrinervis* is the product of inbred *Lamarckiana* and the C.S.H. type the result of crossing one *Lamarckiana* individual with another, unrelated *Lamarckiana*, one should not overlook the fact that de Vries also obtained the Amsterdam type from *O. lata* × *O. nanella*, *O. lata* × *O. brevistylis*, *O. nanella* × *O. brevistylis*, *O. scintillans* × *O. nanella*, *O. lata*, *O. oblonga*, etc.

Since Gates found 14 chromosomes in *rubrinervis* and since the plants in which he made these counts were also grown in America, it would be instructive to learn whether they duplicated the vegetative characters of de Vries's form throughout, or whether they also were modified individuals of the same type as the New York Botanical Garden and Cold Spring Harbor mutants. Had the small chromosome been present in the somatic cells of the plant which Gates studied as it was in the Amsterdam mutant, it is not probable that it would have been overlooked. It will be borne in mind, of course, that it has not yet been shown that all plants having the same type of vegetative characters as de Vries's mutant have 14^{+1} chromosomes; for, thus far, the somatic chromosome number of but one Amsterdam *rubrinervis* has been reported. It may be that the small remnant merely chanced to be handed down from the *lata* mother of No. 6082 without affecting the characters of the *rubrinervis* offspring.

SUMMARY

1. The primary object of this series of three papers, of which the present report is the first, is to discuss, in the light of the Cold Spring Harbor and Louvain studies of somatic chromosome number in *Oenothera Lamarckiana* and its derivatives, certain theories and conclusions which Gates has advanced from time to time, and which Gates and Miss Thomas have based upon the results of their investigations.

2. Five hitherto undescribed types of 14-chromosome mutants have been recognized in Cold Spring Harbor cultures of *O. Lamarckiana*, *O. lata*, *O. nanella*, etc.; namely, types 2787, 2803, 3539, *O. plicatula* and *O. delicatula*. In addition to the foregoing, 14 chromosomes have been counted in a type (3514) figured and described by MacDougal in 1905 as *O. rubrinervis*. This form is believed by the writer to be a modified form of the Amsterdam mutant. It is not known whether the *rubrinervis* in which Gates counted 14 chromosomes (reported in 1908) duplicated the characters of the Dutch, or of the American type.

3. One plant was found in a 1908, and another in a 1909, culture of *O. lata* × *O. Lamarckiana*, each having 14 chromosomes of the usual size and 1 very small one. The two plants had the same type of vegetative character and the mutant form which they represented will be known as *O. aberrans*. The same condition was found in a *rubrinervis* mutant from one of de Vries's 1912 cultures of *O. lata* × *O. Lamarckiana*.

4. In each of the three plants the small chromosome usually, though not invariably, occupied a peripheral position on the equatorial plate and was commonly alloted the full space of an ordinary chromosome.

5. The small chromosome of de Vries's *rubrinervis* differed slightly in appearance from that of *O. aberrans* and the somatic characters of the two forms were strongly contrasted.

6. It was not ascertained in any one of the three plants whether or not the small chromosome invariably accompanied the 14 ordinary chromosomes in the somatic cells examined, but it was clearly demonstrated that it was, at least, seldom absent.

7. Gates and Miss Thomas found that one of the 15 chromosomes of *O. lata* may sometimes fragment and degenerate during male reduction and have presented evidence which indicates that male gametes may be formed containing one or more fragments of chromosomes in addition to the ordinary, whole chromosomes. We have no evidence to show whether 7^{+1}-chromosome male gametes, *capable of functioning*, are produced by any form. However, it is probable that the extra chromosome of 15-chromosome forms sometimes fragments during female reduction and that 7^{+1}-chromosome eggs, capable of functioning, are produced occasionally.

8. Since each of the three 14^{+1}-chromosome mutants was derived from 15-chromosome *O. lata* × 14-chromosome *O. Lamarckiana*, it is probable that all were products of $♀\, 7^{+1} + ♂\, 7$.

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EXPLANATION OF PLATE XXIV.

All figures were drawn with the aid of a camera lucida, Bausch and Lomb 1 inch ocular and oil immersion, ap. 1.9. Figures drawn from sections of root-tips taken from greenhouse rosettes. Cut 7 μ thick and stained with Heidenhain's iron-alum haematoxylin. Figures 1–11 taken from transverse, and 12–13 from longitudinal, sections.

FIG. 1. *O. aberrans*, plant No. 3878, C. S. H., 1908. Mutant offspring of *O. lata* × *O. Lamarckiana*. Polar view of metaphase group showing 14 chromosomes of the usual size and 1 very small one, the latter occupying a peripheral position on the equatorial plate.

FIG. 2. *O. aberrans*, plant No. 4605, C. S. H., 1909. Mutant offspring of *O. lata* × *O. Lamarckiana*. Polar view of metaphase group showing 14^{+1}-chromosomes, the small body lying farther in the group than in the preceding figure.

FIG. 3. *O. aberrans*, plant No. 4605, C. S. H., 1909. Polar view of metaphase group showing 14^{+1}-chromosomes, the small body occupying a peripheral position on the equatorial plate.

FIG. 4. *O. rubrinervis*, plant No. 6082, Amsterdam, 1912. Mutant offspring of *O. lata* × *O. Lamarckiana*. Polar view of metaphase group showing 14^{+1}-chromosomes.

FIG. 5. *O. rubrinervis*, plant No. 6082, Amsterdam, 1912. Same as fig. 4. The small chromosome of figs. 4 and 5 occupies a peripheral position on the equatorial plate in each case. Note also that the small body of this plant appears to be almost round and apparently somewhat broader than the ordinary chromosomes.

FIG. 6. *O. gigas*, plant No. 3676, C. S. H., 1908. Offspring of *O. gigas*. Nuclear prophase stage showing chromosome *a* with clear area near one end.

FIG. 7. *O. Lamarckiana*, plant No. 970, C. S. H., 1907. Offspring of *O. Lamarckiana*. Polar view of metaphase group of 14 chromosomes showing *a* with clear area at the middle of the body.

FIG. 8. *O. Lamarckiana*, plant No. 983, C. S. H., 1907. Offspring of *O. Lamarckiana*. Polar view of metaphase group of 14 chromosomes showing *a* with clear area between middle and extremity of the body.

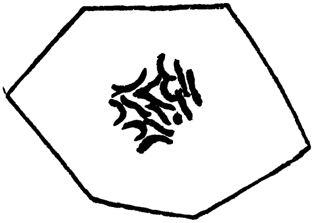
FIG. 9. *O. Lamarckiana*, plant No. 2488, C. S. H., 1908. Offspring of *O. lata* × *O. Lamarckiana*. Polar view of metaphase group of 14 chromosomes showing *a* and *b* each with a clear area near one extremity of the body.

FIG. 10. Chromosome *a*, plant No. 3433, C. S. H., 1908; a 21-chromosome mutant offspring of *O. lata* × *O. Lamarckiana*. Chromosome *b*, plant No. 3627, C. S. H., 1908; *O. albida*, a 15-chromosome mutant offspring of *O. Lamarckiana*. Chromosome *c*, plant No. 1133, 1907; *O. gigas* offspring of *O. gigas*. All taken from polar views of metaphase groups; *a* and *b* each display a single large, clear area, while two such regions may be observed in *c*. Owing to the fact that *a* and *b* are more heavily stained than *c* and than the chromosomes of figs. 6–9, the linin surrounding the clear area in *a* and *b* appears as delicate side-lines connecting the heavily stained portions, while it is invisible in the remaining chromosomes mentioned. The relative positions of the heavily stained regions clearly show that we are dealing with whole chromosomes and not with fragments.

FIG. 11. Type 5509, plant No. 5509. C. S. H., 1908. Supposed to be a modified form of *O. oblonga*. Mutant offspring of *O. Lamarckiana*. Polar view of a metaphase group showing chromosome *a* torn apart in the region of the clear area and the small extremity displaced, probably by the knife in sectioning.

FIG. 12. *O. nanella*, plant No. 2788, C. S. H., 1908. Offspring of *O. nanella*. Lateral view of daughter chromosomes in anaphase showing clear areas in corresponding regions. Note the delicate linin side-lines connecting the heavily stained portions.

FIG. 13. *O. gigas*, plant No. 4662, C. S. H., 1909. Offspring of *O. gigas*. Description the same as for fig. 12.



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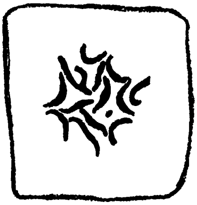

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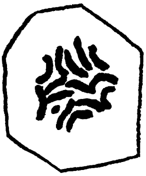

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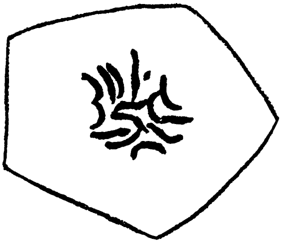

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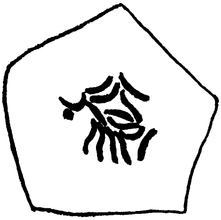

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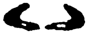

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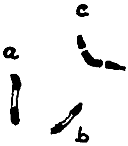

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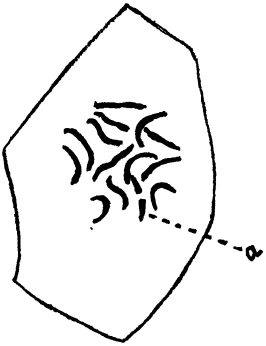

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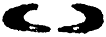

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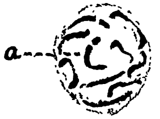

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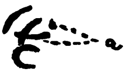

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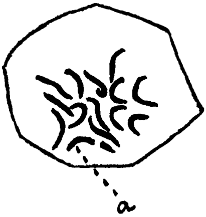

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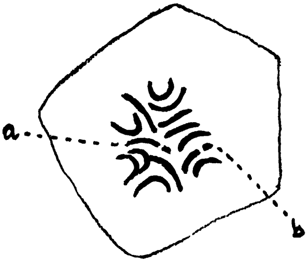

9

LUTZ: DIMINUTIVE CHROMOSOMES IN OENOTHERA.